厚生労働科学研究費

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体脂肪減少因子を用いた２型糖尿病の治療に関する研究

平成２０年度　総括研究報告書

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【目次】

「体脂肪減少因子を用いた２型糖尿病の治療に関する研究」
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【１】総括研究報告

厚生労働省科学研究費補助金（創薬基盤推進研究事業）
総括研究報告書

体脂肪減少因子を用いた２型糖尿病の治療に関する研究

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研究要旨

日本人の２型糖尿病は、膵β細胞のインスリン分泌不全を基盤とし、軽度の肥満によるインスリン抵抗性の付加によって発症リスクが上昇する。膵島トランスクリプトーム研究により、申請者らは、蓄積した体脂肪を減少させると共に、血糖を低下させる分泌蛋白を発見した。本研究では、同蛋白の患者血中測定による糖脂質代謝異常の診断法の開発と治療への応用を目指す。さらに、同蛋白のコード遺伝子多型を用いた疾患発症と治療の感受性の体質診断も試みる。本年度の研究では、血糖降下の機序として膵β細胞のインスリン分泌の増強やインスリン感受性よりも、肝臓での糖取り込みが亢進している可能性が示唆された。また、同蛋白を過剰発現する実験動物の作成にも成功した。

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研究目的

糖尿病に肥満の合併は高頻度であり、治療や予防において肥満の解消は重要である。岐阜市で実施された糖尿病の実態調査（1,070 人を対象とした 75g 経口糖負荷試験）では、日本人の病態はインスリン分泌不全であり、特に初期分泌から障害されて食後高血糖が始まることが確認された。合併する肥満やインスリン抵抗性が軽度であっても、これを補充できるインスリン分泌能が備わっていないので、先ずは肥満の解消による発症予防が重要になる。特に、軽症糖尿病においてもリスクの高い心血管イベントの予防に対しても重要である。しかし、食事療法による減量は長期の努力を要するにも関わらず、十分な改善を見るに至らない場合がほとんどであり、体重増加のリバウンドも多い。運動療法はインスリン抵抗性の改善に欠かせないが、心機能の低下、腎障害、増殖網膜症、関節障害、リハビリなどを有する状態では、運動は困難で適応にない。一方、短期の減量には VLDC 療が行なわれるが、入院を要する上に反動が大きい。食欲低下薬は適応が厳しく、しかも長期使用は認められていない。従って、短期に効率的な体脂肪の減量ができて、しかも血糖改善を同時に取り込める治療は理想的である。我が国が迎える高齢化社会ではこのような治療は患者の生活自立阻害を解消すると期待される。

申請者らは、膵島トランスクリプトーム研究の過程において、発現遺伝子のコード蛋白の内で特に分泌蛋白に焦点を当てた研究を行なってきた。「分泌蛋白」に焦点を当てた理由は、患者の血中測定と投与が容易であり、早期の臨床応用に直結するからである。正常と糖尿病における遺伝子発現プロフィールの比較解析から、幾つかの変化遺伝子に着目した。実験動物の肝で過剰発現させたところ、生理機能が不明な 32kDa 分子が体脂肪量を減少させた。興味深いことに予備実験では血糖値を低下させた。本研究では、32kDa 分子について肥満と糖尿病の双方に関する診断法の開発し、さらに、SNP を用いた体質診断と治療創薬への臨床応用を目指す。

本年度の研究では、血糖降下作用の機序に重点をおいて計画を進めた。

研究方法

血糖値の低下を生じる機序の解析

前年度に、マウスインスリン産生細胞株 MIN6 に過剰発現させ、種々のグルコース濃度に対する分泌量を測定することによってインスリン合成と分泌に対する 32kDa 分子の効果を解析した。インスリン分泌に対する効果は軽微であったので、GTT における血糖降下現象を説明することは困難であった。そこで、インスリン分泌と独立して末梢での糖の取り込みが促進する可能性を検討した。すなわち、インスリン感受性の変化と肝臓の関与に対する検討である。

ヒトでも前年度までに血中測定系を開発しているので、糖尿病が疑われる症例数を前年度よりも増やして７５g 糖負荷試験を継続して実施し、耐糖能を３群（正常型、境界型、糖尿病型）に区分して各々のグループで血糖パターンとインスリン分泌パターンとの関連を解析した。

遺伝子多型と病態との関連解析

前年度までに、32kDa 分子のコード遺伝子のエクソンと周辺領域を全シーケンスして頻度の高い一塩基多型（SNP）を獲得し、SNP ハプロタイプを利用して２型糖尿病との関連解析を実施してきた。当該年度は、血中レベルと相関するマーカーを検討した。

倫理面への配慮

全ての実験はヘルシンキ宣言と３省庁合同指針を遵守して行われる。本計画は医学部の遺伝子解析と臨床研究に関する倫理審査委員会の承認を既に受けている。患者および健常者からの DNA と血清試料はインフォームドコンセントを取得した後に提供を受け、連結可能匿名化の状態で保存されている。匿名化 DNA はさらにプレート番号のみで管理されるので二重に匿名化され、被験者のプライバシーは完全に保護される。臨床上の個人情報を含めて、研究ソースはすべて本研究に関わらない秘守義務を負う識別管理者（岐阜大学医学部の倫理委員会が指定する者）が管理する。保存コンピュータはインターネットに連結せず、専用で独立である。

C　研究結果

血糖値の低下を生じる機序の解析

正常ラットを用いた糖負荷試験では、前、30 分、120 分のいずれにおいても血糖低下が再現された。前年度は対応するインスリン分泌については試料が十分ではなく測定できなかったが、本年度は個体数を増やすことで検討することができた。その結果、インスリン合成と分泌には有意な変化を認めなかった（Ad-GFP 前 0.24±0.05、30 分 0.43±0.11、vs　Ad-32kDa 前 0.27±0.05、30 分 0.4±0.08、NS)。血糖降下作用はインスリン分泌とは直接的に関連しないものと結論された。本年度は

餌負荷における血糖の変化についても検討した。この結果、空腹時（対照 108±10 vs 32kDa 67±4, p<0.05）も ad. lib. Fed（対照 132±17 vs 32kDa 03±16, p<0.05）のいずれにおいても有意に血糖値低下した。一方、インスリン負荷による末梢組織の感受性についても検討したが、過剰発現にて有意差は認めなかった。

前年度に HbA1c 5.5 以上のヒト 78 人について 5g 経口糖負荷試験を実施したところ、糖尿病型では全時点で低分泌が観察された。境界型と糖尿病型の症例数が十分ではなかったので、本年度はさらに症例数（188 人）を増やして検討を加えた（正常型 68 人、境界型 21 人、糖尿病型 99 人）。この結果、糖尿病にて有意な血中レベルの低下を認めた（p<0.05）。

肝における糖代謝の解析

肝臓は食事由来の門脈血のグルコースを取り込む。一方、空腹時にはグリコーゲンを分解して糖新生を行い、血糖の恒常性を維持している。糖負荷試験で血糖降下を説明できるほどのインスリン分泌の亢進は認められなかったので、肝における糖の取り込みの影響が示唆された。そこで、糖代謝に関連する分子の転写レベルでの変化を解析した。各々の mRNA を前値と相対比較すると、解糖系の酵素は有意に亢進し（GK 2.25±0.09）、糖新生系の酵素は有意に減少していた（PEPCK 0.33±0.01）。一方、肝のグリコーゲンの蓄積量は有意に減少していた（対照 118±13 vs 32kDa 73.6±23.7, p<0.05）。

脂肪代謝に対する効果の解析

32kDa 分子の過剰発現により、体脂肪蓄積は減少したが、本年度は個体数を増やして脂質レベルに対する影響を解析した。血中の中性脂肪は空腹時（対照 129±24 vs 32kDa 156±36, p<0.05）と ad. lib. Fed（対照 149±20 vs 32kDa 176±29, p<0.05）のいずれにおいても有意に上昇した。しかしながら、遊離脂肪酸は空腹時（対照 225±0.09 vs 32kDa 2.70±0.19, p<0.05）においてのみ有意差を認め食後には関連を認めなかった。肝臓の中性脂肪の蓄積量は有意に増加していた（対照 10.3±1.9 vs 32kDa 33.0±9.9, p<0.05）。

脂質代謝に関連する分子の転写レベルでの変化を解析した。各々の mRNA を前値と相対比較すると、脂質合成と分解に関しては、糖代謝と異なり、有意な変化は認めなかった（ACC2, CPT1, ACOX, ACC, FAS）。

前年度はアデノウイルスの発現系を用いて腹腔内に投与し、肝臓から高分泌させた解析を行ったが、ウイルスそのものの影響の関与が示唆された。そこで、本年度は過剰発現する TG マウスの作成を試みた。現在、ファウンダー５系統が得られているが、著明な肥満ややせの個体は認めていない。この点はアデノウイルス発現系を用いた結果と同様であった。次年度に生理学的にモデル動物の代謝システムを総合的に解析する予定である。

遺伝子多型を用いた関連解析

32kDa 分子のコード遺伝子の 10 エクソンを直接シーケンスで解析したが、家族性糖尿病（MODY）の原因となる変異は同定されなかった。次いで、全域を SNP スクリーニングして頻度の高い SNP を前年度よりさらに 6 個見出した（合計 18 個）。昨年度の解析から、連鎖不平衡解析により既に２つの LD ブロックを見出している。代表 SNP を用いて 32 kDa 分子の血中レベルとの相関解析の結果、イントロン８に存在する SNP-136 において有意の相関を認めた（p=0.011）。この差は女性において大きい傾向が見られたが、性差の確認にはさらに症例を増やすことによる確認が必要である。

考察

インスリン抵抗性の改善のために、２型糖尿病の予防と治療の双方に過体重の解消は重要である。動脈硬化による心血管イベントの予防にも解消は欠かせない。しかし、食事療法と運動療法を中心とした減量は不断の長期努力を要するので、続かず十分な改善に至らない場合がほとんどである。特に高齢者では、循環器疾患を既に合併していたり、整形外科的な障害のために運動制限がある場合が多い。従って、効果的な減量と耐糖能の改善を同時に見込める治療は理想的である。特に、継続的な運動やインスリン治療が困難な高齢化社会では、このような治療法は老人の生活自立阻害を予防する。

一方、内蔵肥満があると軽度の耐糖能障害であっても、心血管イベントリスクとなることが明らかにされている。従って、壮年期からの健康診断による効率歴なリスクグループの早期検出と保健指導は予防策として重要である。75g 糖負荷試験は耐糖能異常を検出する鋭敏な検査法であるが、少なくとも２時間を要すること、費用、煩雑な手間から一次健診には適するとは言えない。32 kDa 分子の随時の１回採血で高リスク者が検出されれば健診効率は上昇すると期待される。

結論

32 kDa 分子は主として肝臓の糖代謝に作用して血糖値を低下させる可能性が考えられた。インスリンの初期分泌不全とインスリン抵抗性に次いで、食後高血糖の第３の可能性が提起された。32 kDa 分子の適正な血中レベルの調節は理にかなった２型糖尿病の治療であるかもしれない。

健康危険情報

なし

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H　知的財産権の出願・登録

1　特許取得

なし

2　実用新案登録

なし

3　その他

なし

【２】研究成果の刊行に関する一覧表

雑誌

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K. Izuka and Y. Horikawa.	ChREBP: A glucose-activated transcription factor involved in the development of metabolic syndrome.	Endocrine Journal	55	617-624	2008
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E. Kuroda, et al.	Identification of minimal promoter and genetic variants of Kruppel-like factor 11 gene and association analysis with type 2 diabetes in Japanese.	Endocrine Jpurnal		In press	2009

【３】研究成果の刊行物・別刷

Replication of Genome-Wide Association Studies of Type 2 Diabetes Susceptibility in Japan

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Background: In Europeans and populations of European origin, several groups have recently identified novel type 2 diabetes susceptibility genes, including *FTO*, *SLC30A8*, *HHEX*, *CDKAL1*, *CDKN2B*, and *IGF2BP2*, none of which were in the list of functional candidates.

Objective and Design: The aim of this study was to replicate in a Japanese population previously identified associations of single nucleotide polymorphisms (SNPs) within 10 candidate loci with type 2 diabetes using a relatively large sample size: 1921 subjects with type 2 diabetes and 1622 normal controls.

Results: A total of 15 SNPs were genotyped. Eight SNPs in five loci were found to be associated with type 2 diabetes: rs3802177 [odds ratio (OR) = 1.16 (95% confidence interval (CI) 1.05–1.27); $P = 4.5 \times 10^{-3}$] in *SLC30A8*; rs1111875 [OR = 1.27 (95% CI 1.14–1.40); $P = 1.4 \times 10^{-5}$] and rs7923837 [OR = 1.27 (95% CI 1.13–1.43); $P = 1.0 \times 10^{-4}$] in *HHEX*; rs10811661 [OR = 1.27 (95% CI 1.15–1.40); $P = 1.9 \times 10^{-6}$] in *CDKN2B*; rs4402960 [OR = 1.23 (95% CI 1.11–1.36); $P = 8.1 \times 10^{-5}$] and rs1470579 [OR = 1.18 (95% CI 1.07–1.31); $P = 8.3 \times 10^{-4}$] in *IGF2BP2*; and rs7754840 [OR = 1.28 (95% CI 1.17–1.41); $P = 4.5 \times 10^{-7}$] and rs7756992 [OR = 1.27 (95% CI 1.15–1.40); $P = 9.8 \times 10^{-7}$] in *CDKAL1*. The first and second strongest associations were found at variants in *CDKAL1 and CDKN2B*, both of which are involved in the regenerative capacity of pancreatic β-cells.

Conclusion: Some of these variants represent common type 2 diabetes-susceptibility genes in both Japanese and Europeans. (***J Clin Endocrinol Metab* 93: 3136–3141, 2008**)

Type 2 diabetes is a complex disease with several genes and environmental factors involved in onset and development. To date, a number of genes have been reported to be associated with type 2 diabetes. Most of these were investigated because of their assumed relevance to the pathogenesis of type 2 diabetes based on their functions. However, because the pathogenesis of

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Abbreviations: BMI, Body mass index; *CDKAL1*, cyclin-dependent kinase inhibitor 5 regulatory subunit associated protein 1-like 1 gene; *CDKN2B*, cyclin-dependent kinase inhibitor 2B gene; CI, confidence interval; *EXT2*, exostosin 2; *FTO*, fat mass and obesity associated gene; *GCKR*, glucokinase regulatory protein gene; *HHEX*, hematopoietically expressed homeobox gene; HOMA, homeostasis model assessment; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; *IGF2BP2*, IGF2 mRNA binding protein 2 gene; LD, linkage disequilibrium; OR, odds ratio; *SLC30A8*, zinc transporter gene; SNP, single nucleotide polymorphism; TG, triglyceride.

type 2 diabetes is yet to be elucidated completely, the candidate-gene approach is limited in power to detect novel disease-susceptibility genes. A strongly associated type 2 diabetes gene, transcription factor 7-like 2, has been identified by a genome-wide linkage study (1). Several groups confirmed a significant association between type 2 diabetes and this gene in various populations, with some noteworthy exceptions (2–7). Genome-wide association studies using 300,000–500,000 single nucleotide polymorphisms (SNPs) and high throughput technology overcome the limitation of function-based investigation, and novel susceptibility genes for type 2 diabetes, including zinc transporter (*SLC30A8*), hematopoietically expressed homeobox (*HHEX*), cyclin-dependent kinase inhibitor 2B (*CDKN2B*), IGF2 mRNA binding protein 2 (*IGF2BP2*), and CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), have recently been identified. In addition, the fat mass and obesity associated gene (*FTO*) and glucokinase regulatory protein gene (*GCKR*) were associated with body mass index (BMI) and serum triglyceride (TG) level, respectively (8–13). All of these proven genes for type 2 diabetes have been reproducibly associated in multiple studies (14). Meanwhile, exostosin 2 (*EXT2*), *LOC387761* (11), and an intergenic signal (rs9300039) (9) were identified in a single study and have not been replicated. However, most of the populations analyzed were of European ancestry, except in the case of *CDKAL1*, which was replicated in subjects from Hong-Kong. To distinguish variants that are common and reproducible susceptibility genes, it is important to replicate the associations of candidate SNPs with type 2 diabetes in various ethnic groups. In this study we examined the association of recently identified risk SNPs in 10 candidate loci with type 2 diabetes in a relatively large sample set of Japanese subjects.

Subjects and Methods

Subjects

Three sample sets were involved. The Kobe set and the Gunma set subjects were recruited from hospitals in Hyogo and Gunma prefecture, respectively. The Consortium set subjects were recruited from seven districts in Japan by the Study Group of the Millennium Genome Project for Diabetes Mellitus. The inclusion criteria for normal, control subjects of these three sets were as follows: 1) older than 60 yr, 2) glycosylated hemoglobin A_{1c} values less than 5.8%, and 3) no past history of type 2 diabetes. Type 2 diabetes was diagnosed in accordance with World Health Organization criteria. Other forms of diabetes were excluded based on the clinical data. The clinical and laboratory characteristics of the study subjects are shown in supplemental Table 1, which is published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. Written, informed consent was obtained from all participants. This study was approved by the ethics committee of each participating institute (6).

Genotyping

There were 15 SNPs genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). These SNPs were selected based on previous reports (8–13) and HapMap linkage disequilibrium (LD) data of Japanese. Departures from Hardy Weinberg Equilibrium were defined as $P < 0.001$ in cases and controls (11). Because SNP rs13266634 in *SLC30A8* was deviated from Hardy Weinberg Equilibrium, SNP rs3802177 in the same gene, which is in strong LD with rs13266634 ($r^2 = 0.96$, HapMap LD data of Japanese), was also examined. The genotyping success rate in the three sample sets was more than 96%. The genotypes determined by TaqMan methods were identical to those determined by direct sequencing for 48 samples. The risk allele of each SNP is shown in supplemental Table 2.

Clinical assessment

The clinical profile of each subject was directly determined at entry. Association studies were performed between the candidate SNPs and BMI, homeostasis model assessment (HOMA) [HOMA of insulin resistance (HOMA-IR) and HOMA of β-cell function (HOMA-β)], or serum TG level. Subjects who had not been treated with insulin were evaluated for HOMA-IR and HOMA-β. Data are expressed as means ± SD.

Statistical analysis

The differences in SNPs between type 2 diabetic and nondiabetic subjects were compared using χ^2 test and multiple logistic regression analysis under additive, dominant, and recessive models for SNPs. The Cochran-Armitage trend test was also performed with the additive model. There was no heterogeneity among the samples in regard to the recruiting districts. We considered statistical significance at P values less than 0.0033 (0.05/15) in the association study for SNPs after Bonferroni correction. The relation of the variants in these genes with BMI, HOMA-IR, HOMA-β, and TG was assessed by ANOVA for each SNP. The HOMA-IR, HOMA-β, and TG data were log transformed for normality. Statistical analysis was performed with the StatView program (version 5.0-J; SAS Institute Inc., Cary, NC). LD analysis was performed with Haploview (http://www.broad.mit.edu/personal/jcbarret/haploview).

Results

There were 15 SNPs from 10 candidate loci examined for association with type 2 diabetes with a criterion of significance of P value less than $0.05/15 = 0.0033$ after Bonferroni adjustment. Eight SNPs in five loci, *SLC30A8* (rs3802177), *HHEX* (rs1111875, rs7923837), *CDKN2B* (rs10811661), *IGF2BP2* (rs4402960, rs1470579), and *CDKAL1* (rs7754840, rs7756992), were found to be associated with the occurrence of type 2 diabetes (Tables 1 and 2). The P values of association for *CDKN2B* (rs10811661) and *CDKAL1* (rs7754840 and rs7756992) were about 1.9×10^{-6}, 4.5×10^{-7}, and 9.8×10^{-7}, respectively. The next strongest association was found for *IGF2BP2* (rs4402960 and rs1470579) and *HHEX* (rs1111875 and rs7923837) at a P value of 10^{-4}–10^{-5}. SNP rs3802177 of *SLC30A8* showed a nominal association, which disappeared after adjustment for age, sex, and BMI. No association of the other SNPs with type 2 diabetes was detected.

Association studies were also performed between *FTO* and BMI and *GCKR* and serum TG level using the samples with serum data according to previous reports. A nominal association of *GCKR* (rs780094) with serum TG level both in case and control subjects was found in our samples, as previously reported in Caucasians (10). In control subjects the mean values of serum TG were 1.07 ± 0.53, 1.13 ± 0.49, and 1.18 ± 0.55 mmol/liter for CC, CT, and TT genotype, respectively ($P = 0.097$). In cases, these values were 1.32 ± 0.73, 1.43 ± 1.57, and 1.56 ± 1.05 mmol/liter for CC, CT, and TT genotype, respectively ($P = 0.063$). Association of the SNP of *FTO* (rs9939609) with BMI

TABLE 1. Association results between 15 SNPs in 10 candidate loci and type 2 diabetes in Japanese

Gene	SNP ID	Genotype T2DM			Genotype CONT			RAF						95% CI			
		RR	Rr	rr	RR	Rr	rr	T2DM	CONT	*P* value	Armitage trend	*P* value[a]	OR	Upper	Lower	RAF-C	OR -C
SLC30A8	rs13266634	690	806	334	522	725	327	0.60	0.56	3.2×10^{-3}	4.5×10^{-3}	0.17	1.16	1.05	1.27	0.65	1.12
SLC30A8	rs3802177	649	885	306	473	808	291	0.59	0.56	3.2×10^{-3}	3.0×10^{-3}	0.065	1.16	1.05	1.27		
HHEX	rs1111875	212	784	852	132	603	828	0.33	0.28	9.6×10^{-6}	1.4×10^{-5}	8.4×10^{-5}	1.27	1.14	1.40	0.53	1.13
HHEX	rs7923837	98	633	1113	60	467	1049	0.22	0.19	8.8×10^{-5}	1.0×10^{-4}	6.7×10^{-3}	1.27	1.13	1.43	0.62	1.22
LOC387761	rs7480010	1226	556	68	1018	481	67	0.81	0.80	0.33[a]	0.33[a]	0.29	1.06	0.94	1.20		
EXT2	rs3740878	260	842	731	211	738	629	0.37	0.37	0.73[a]	0.74[a]	0.63	1.02	0.92	1.12		
CDKN2B	rs10811661	683	891	283	486	770	326	0.61	0.55	1.7×10^{-6}	1.9×10^{-6}	5.8×10^{-6}	1.27	1.15	1.40	0.83	1.20
CDKN2B	rs564398	1342	482	47	1122	416	41	0.85	0.84	0.67[a]	0.67[a]	0.65	1.03	0.90	1.17	0.56	1.12
GCKR	rs7800944	421	903	534	312	782	492	0.47	0.44	0.029[a]	0.030[a]	0.017	1.11	1.01	1.22		
Inter gene	rs9300039	1068	684	105	903	565	111	0.76	0.75	0.41[a]	0.42[a]	0.15	1.05	0.94	1.17		
IGF2BP2	rs4402960	230	835	787	143	675	759	0.35	0.30	7.9×10^{-5}	8.1×10^{-5}	9.4×10^{-4}	1.23	1.11	1.36	0.29	1.14
IGF2BP2	rs1470579	260	874	738	165	735	694	0.37	0.33	9.0×10^{-4}	8.3×10^{-4}	2.8×10^{-3}	1.18	1.07	1.31	0.30	1.17
CDKAL1	rs7754840	446	881	543	262	781	538	0.47	0.41	3.2×10^{-7}	4.5×10^{-7}	3.5×10^{-7}	1.28	1.17	1.41	0.31	1.12
CDKAL1	rs7756992	537	876	442	330	818	438	0.53	0.47	8.0×10^{-7}	9.8×10^{-7}	3.9×10^{-6}	1.27	1.15	1.40	0.26	1.20
FTO	rs9939609	88	596	1165	63	520	995	0.21	0.20	0.68[a]	0.68[a]	0.74	1.03	0.91	1.15		

ORs, 95% CIs, and *P* values are given for 15 SNPs identified in French, decode, Diabetes Genetics Initiative, Wellcome Trust Case Control Consortium, and Finland-United States Investigation of Noninsulin-Dependent Diabetes Mellitus Genetics studies. SNPs are shown with the risk allele (R) and risk allele frequency (RAF) and the exact count of each genotype in type 2 diabetic (T2DM) patients and controls (CONT). Risk allele-specific ORs and *P* values were calculated using an additive genetic model that in logistic regression is multiplicative on the OR scale. $r^2 = 0.83$ (rs13266634 and rs3802177), 0.22 (rs1111875 and rs7923837), 0.001 (rs10811661 and rs10811661), 0.87 (rs4402960 and rs1470579), and 0.69 (rs7754840 and rs7756992) in controls of this study. ID, Identification; OR-C, OR in Caucasians; r, nonrisk allele; RAF-C, risk allele frequency in Caucasian controls.

[a] *P* values adjusted for age, sex, and BMI.

TABLE 2. Association results between 15 SNPs in 10 candidate loci and type 2 diabetes in Japanese

		Dominant model					Recessive model				
					95% CI					95% CI	
Gene	SNP ID	*P* value	*P* value[a]	OR	Lower	Upper	*P* value	*P* value[a]	OR	Lower	Upper
SLC30A8	rs13266634	0.063	0.24	1.17	0.99	1.39	5.8×10^{-3}	0.074	1.22	1.06	1.40
SLC30A8	rs3802177	0.15	0.36	1.14	0.95	1.36	1.3×10^{-3}	0.020	1.27	1.10	1.46
HHEX	rs1111875	6.4×10^{-5}	1.6×10^{-5}	1.32	1.15[a]	1.51	3.5×10^{-3}	0.083	1.40	1.12	1.77
HHEX	rs7923837	1.8×10^{-4}	1.9×10^{-3}	1.31	1.14[a]	1.50	0.036	0.15	1.42	1.02	1.97
LOC387761	rs7480010	0.37	0.16	1.17	0.83[a]	1.65	0.44	0.28	1.06	0.92	1.22
EXT2	rs3740878	0.99	0.66	1.00	0.87[a]	1.15	0.49	0.50	1.07	0.88	1.30
CDKN2B	rs10811661	4.0×10^{-5}	2.3×10^{-5}	1.44	1.21[a]	1.72	1.9×10^{-4}	1.8×10^{-4}	1.31	1.14	1.51
CDKN2B	rs564398	0.88	0.51	1.03	0.68[a]	1.58	0.66	0.65	1.03	0.89	1.20
GCKR	rs7800944	0.14	0.34	1.12	0.96[a]	1.29	0.033	4.2×10^{-3}	1.20	1.01	1.41
Inter gene	rs9300039	0.098	0.20	1.26	0.96[a]	1.66	0.85	0.32	1.01	0.88	1.16
IGF2BP2	rs4402960	9.5×10^{-4}	0.018	1.26	1.10[a]	1.44	1.7×10^{-3}	4.9×10^{-4}	1.42	1.14	1.77
IGF2BP2	rs1470579	0.014	0.063	1.18	1.03[a]	1.36	1.6×10^{-3}	9.2×10^{-4}	1.40	1.13	1.72
CDKAL1	rs7754840	1.6×10^{-3}	1.6×10^{-3}	1.26	1.09[a]	1.46	1.3×10^{-7}	1.5×10^{-7}	1.58	1.33	1.87
CDKAL1	rs7756992	0.01	0.022	1.22	1.05[a]	1.42	4.2×10^{-8}	7.4×10^{-7}	1.55	1.32	1.82
FTO	rs9939609	0.98	0.60	1.00	0.87[a]	1.15	0.28	0.70	1.20	0.86	1.67

ORs, 95% CIs, and *P* values under a dominant or recessive model of each risk allele are given for 15 SNPs identified in French, decode, Diabetes Genetics Initiative, Wellcome Trust Case Control Consortium, and Finland-United States Investigation of Noninsulin-Dependent Diabetes Mellitus Genetics studies. ID, Identification.

[a] *P* values adjusted for age, sex, and BMI.

was found only in control subjects. In addition, subjects with the risk A allele tended to show a larger BMI, as previously reported in Caucasians (13), although it did not reach the level of statistical significance. The mean values of BMI were 22.4 ± 3.2 (TT) and 22.7 ± 3.1 kg/m^2 (AA + AT) for controls ($P = 0.051$). On the other hand, these values were 23.6 ± 3.4 (TT) and 23.8 ± 3.8 kg/m^2 (AA + AT) for cases ($P = 0.306$). Although *HHEX*, *CDKN2B*, *IGF2BP2*, and *CDKAL1* were associated with pancreatic β-cell function in recent reports (15–17), we failed to detect an association with HOMA-β in this study. There was also no evidence of association between HOMA-IR and the risk alleles of these genes.

Discussion

Recent reports have revealed novel type 2 diabetes-susceptibility genes such as *SLC30A8*, *HHEX*, *CDKN2B*, *IGF2BP2*, and *CDKAL1* in the European population (8, 9, 11, 12). In addition, *FTO* and *GCKR* were associated with BMI and serum TG level, respectively (10, 13). In this study we confirmed that all of the proven genes found in Caucasians are replicated in Japanese. The strongest association by *P* value at the 10^{-6}–10^{-7} level was found at *CDKN2B* (rs10811661) and *CDKAL1* (rs7754840, rs7756992), followed by *HHEX* (rs1111875, rs7923837) and *IGF2BP2* (rs4402960, rs1470579). The odds ratio (OR) values of the first three SNPs were 1.27, 1.28, and 1.27, respectively, an even stronger association than that found in the original genome-wide association study in Europeans (10, 14). There were considerable differences in the frequencies of the risk alleles (Table 1), resulting in difficulty of replication due to decreased power of the study in addition to that due to population difference. According to the previous report in Japanese, the SNPs in *HHEX* showed the strongest association with type 2 diabetes, although the frequencies of risk alleles of SNPs in *HHEX* were even lower in the Japanese samples than European populations (15, 18). The previous report showed significant association with both SNPs in *HHEX* but not with the SNPs in *IGF2BP2* (15). This discrepancy cannot be explained by the small sample number because the risk allele frequencies of *IGF2BP2* are higher in Japanese than Europeans, in contrast to those of *HHEX*.

Although the previous study did not detect association of *IGF2BP2* with type 2 diabetes (15), the gene was detected as a diabetes-susceptibility gene in the present study. The absence of significant association in the previous study may be due to the lack of power deriving mainly from the small sample number. Recently, another study in Japanese has reported that rs4402960 in *IGF2BP2* showed the strongest association with type 2 diabetes, using a larger number of samples (19). The present study had 86% power to detect an OR of 1.20 when the frequency of a risk allele was 35% (rs4402960) and the *P* value was less than 0.0033. However, it is important to note that association study is dependent on discrimination of case and control subjects. It also has been reported that lifestyle changes can reduce the risk of type 2 diabetes, even in individuals carrying the type 2 diabetes-susceptibility variant of *TCF7L2* (20).

CDKAL1 and *CDKN2B* showed a nominal association with type 2 diabetes in a previous report in Japanese (15, 19). SNP rs7756992 in *CDKAL1* has been associated with type 2 diabetes in Han Chinese individuals from Hong Kong (12). A strong association between this SNP and type 2 diabetes [OR = 1.27 (95% confidence interval (CI) 1.15–1.40); $P = 9.8 \times 10^{-7}$] was detected in this study. Because type 2 diabetes in Asians is characterized primarily by β-cell dysfunction, these two genes might well be involved in transduction of glucose toxicity or regenerative capacity of pancreatic β-cells and, thus, are possible susceptibility genes for Japanese type 2 diabetes.

We found a nominal association of *GCKR* (rs780094) with the serum TG level both in case and control subjects, as previously reported in Caucasians (10). A nominal association of the

SNP of *FTO* (rs9939609) with BMI was found only in control subjects. In addition, the subjects with risk A allele showed somewhat larger BMI values, as has been reported in Caucasians (13). *FTO* was identified as a type 2 diabetes-susceptibility variant that predisposes to diabetes in the United Kingdom population through its effect on BMI. The lack of association between BMI and the *FTO* SNPs in Japanese could be due to the fact that our samples were from a less obese population.

In conclusion, we were able to replicate a significant association with the largest number of samples so far in Japanese between type 2 diabetes and SNPs in *SLC30A*, *HHEX*, *CDKN2B*, *IGF2BP2*, and *CDKAL1*, which suggests that these variants represent common type 2 diabetes-susceptibility genes in both Japanese and Europeans. Further investigation is required to identify the most likely functional variants.

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Effect of fasting on PPARγ and AMPK activity in adipocytes

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ABSTRACT

We investigated the effects of fasting on gene expression and intracellular signals regulating energy metabolism in adipose tissue. Following fasting for 15 h or 39 h, epididymal fat pads were isolated from Wistar rats. PPARγ mRNA levels decreased in the adipose tissues isolated from rats fasted for 39 h, whereas adipocyte lipid-binding protein (aP2) and lipoprotein lipase (LPL) mRNA levels increased. Overnight fasting increased the AMP/ATP ratio and AMP-activated protein kinase (AMPK) in adipose tissue, but not in muscle or liver tissue. In addition, the effect of 5-aminoimidazole-4-carboxyamide-ribonucleoside (AICAR) on PPARγ expression in primary cultured adipocytes was investigated. AICAR reduced PPARγ mRNA levels but increased aP2 and LPL mRNA levels. Thus, fasting-induced AMPK activation may affect on the regulation of gene expression in adipocytes.

1. Introduction

Obesity is a major health problem commonly associated with insulin resistance, type 2 diabetes, hypertension, dyslipidemia and atherosclerosis. Numerous studies have demonstrated that elevating PPARγ activity with thiazolidinediones improves insulin sensitivity [1]. On the other hand, little is known about the effects of nutritional state, such as a low calorie diet, on the expression and activity of PPARγ in adipose tissue. Herein, we evaluated the expression of PPARγ and related genes in adipose tissue from fasted animals, and assessed the signals regulating glucose and lipid homeostasis in adipose tissue. In particular, we focused on the relationship between fasting and AMP-activated protein kinase (AMPK) in adipocytes.

AMPK is a heterotrimeric enzyme consisting of a catalytic α subunit and regulatory β and γ subunits [2]. AMPK is activated following an increase in the AMP/ATP ratio rather than the intracellular depletion of ATP or accumulation of AMP [3]. Therefore, AMPK is regarded as a fuel gauge whose activation inhibits ATP-consuming pathways. AMPK has emerged as a key regulator of glucose metabolism. Exercise-induced recruitment of Glut4 to the plasma membrane followed by glucose uptake is mediated via AMPK [4]. Thus, considerable work has elucidated the significance of AMPK in glucose and fat homeostasis and its possible usefulness as a therapeutic target in type 2 diabetes. However, little information is available surrounding its roles in adipose tissue, compared with liver, skeletal muscle. Therefore, we propose a possible role for AMPK in adipocytes isolated from fasted animals.

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